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SHORT COMMUNICATIONS

Microsatellite Markers Reveal Unprecedented High Frequencies of Hybridization among *Typha* Species in the Midwestern US

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Abstract

In North American wetlands, two cattail species -native *Typha latifolia* and exotic *T. angustifolia*- hybridize generating *T. x glauca*. *Typha angustifolia* and the hybrid spread invasively, negatively affecting wetlands. Due to high trait variability and hybridization, *Typha* species are difficult to identify morphologically. Building on previous work that relied on microsatellite markers to differentiate *Typha* species (including hybrids, parental backcrosses, and advanced-generation hybrids) in southern Canada and in the US upper Midwest and northeast, our goals were to 1) estimate relative frequencies of parental species in additional Midwestern cattail populations, and 2) quantify their hybridization. We also assessed level of agreement between morphological identification based on leaf width and gap between inflorescences and molecular identification. Using 6 microsatellites markers (4 used previously in other populations and 2 novel ones), we identified ~25% of the samples as native *T. latifolia,* while ~6% were exotic *T. angustifolia.* Furthermore, 19% of the samples were first-generation hybrids (*T. x glauca*) and 50% were advanced-generation hybrids, with backcrosses to native *T. latifolia* being almost twice as high as those to exotic *T. angustifolia*, rates that are much larger than previously reported. Agreement between morphological and molecular identification was lower than expected highlighting the fact that these morphological traits can be misleading when used alone in cattail identification. We caution that the seemingly asymmetric hybridization towards the native *Typha latifolia* could potentially lead to its extinction in the Midwest. Cattail management may thus require efforts to preserve the native cattail through seed banking and/or other approaches.

Keywords Hybridization · Cattails · *Typha latifolia* · *Typha angustifolia* · *Typha x glauca* · Extinction · Microsatellites

Introduction

In North America, two species of cattails occur widely in Midwestern wetlands: *Typha latifolia,* native to the US, and *T. angustifolia*, a presumably exotic cattail from Europe (Ciotir et al. 2013b; Ciotir and Freeland 2016) but whose origin has been debated (e.g., Pederson et al. 2005; Shih and Finkelstein 2008; Rippke et al. 2010). The two cattail species can hybridize, producing *T. x glauca*, which is more aggressive in the invasion process than exotic *T. angustifolia* (Smith 1987; Travis et al. 2010, 2011; Bunbury-Blanchette et al. 2015; Zapfe and Freeland 2015). Although earlier work on cattail hybrids suggested they were mostly sterile (Smith 1967, 1986) or that the potential for hybridization was low (Selbo and Snow 2004), molecular studies of the *Typha* species group document that *T. x glauca* individuals are fertile, and can backcross to either parental species (*T. latifolia* or *T. angustifolia*) or other hybrids producing advanced-

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generation hybrids (e.g., Kuehn et al. 1999; Snow et al. 2010; Travis et al. 2010; Kirk et al. 2011; Freeland et al. 2013).

Previous studies have studied cattail hybridization in southern Canada, Maine, and Massachusetts (Kuehn et al. 1999; Kuehn and White 1999; Kirk et al. 2011; Freeland et al. 2013; Ciotir et al. 2013a), and in the US upper Midwest and northeast, including sites in five National Parks in Indiana, Michigan, Minnesota, Ohio, and Wisconsin, and other populations in Connecticut, Maryland, Michigan, Minnesota, and New York (Snow et al. 2010; Travis et al. 2010, 2011; Marburger and Travis 2013). These studies documented cattail hybridization and introgression in these areas, yet estimates of hybridization events in more central Midwestern areas (i.e., southern and western to the areas sampled in the studies above) are limited. In addition, hybridization events and hybrid invasiveness may differ in prevalence depending on geographic region (Tsyusko et al. 2005; Travis et al. 2010, 2011; Kirk et al. 2011; Freeland et al. 2013; Marburger and Travis 2013; Ciotir et al. 2017). Thus, better assessment of relative frequencies of *Typha* species and hybridization events in areas that have not been previously sampled could help continue to elucidate the role of hybridization in *Typha* expansion throughout North America.

Due to hybridization, identifying *Typha* species morphologically is difficult because of major phenotypic overlap in these traits across species. Morphological characters commonly used in cattail species identification involve leaf width or gap size between male and female inflorescences (e.g., Kuehn and White 1999). Additionally, pollen grains (Finkelstein 2003; Lishawa et al. 2013; Marburger 2013), shoot anatomy (McManus et al. 2002), and compound pedicel and stigma morphology (Hotchkiss and Dozier 1949; Smith 1986; Kuehn and White 1999) have also been used to differentiate among species and hybrids. However, leaf width and gap between inflorescences remain the most practical and widely used characters in the field due to the lack of need for special equipment or procedures (e.g., microscopes, sample preparation, plant tissue staining and sectioning). Given that *Typha* species can spread extremely quickly by aggressively invading many US wetlands (Tuchman et al. 2009; Bansal et al. 2019) and that they are considered one of the four worst wetland plant invaders in the Midwest (Galatowitsch et al. 1999), molecular tools like microsatellite markers that aid in properly identifying cattail species and their hybrids have been instrumental in forwarding our knowledge about *Typha* dynamics in North America.

We built on the work of Snow et al. (2010), Travis et al. (2010), and Kirk et al. (2011), who used molecular markers (RAPDs, microsatellites) to successfully identify parental cattail species and their hybrids in areas of North America. Our goal was to assess if microsatellite markers used in these studies as well as novel markers from *Typha minima* were useful in discriminating among cattail genotypes in novel populations from seven Midwestern states that had not been sampled before. Additionally, we determined the relative frequencies of parental versus hybrid cattail genotypes, and evaluated if there was evidence of introgression (i.e., backcrosses from the hybrid cattail to the parental species or to other hybrids). Lastly, we also addressed whether and how molecular identification using microsatellites corresponded with morphological identification based on cattail leaf width and gap between inflorescences.

Materials and Methods

During 2012–2013, we collected cattail plant material opportunistically after provisional taxonomic identification using gap between male and female inflorescences and leaf width (no gap (0–5 mm), leaf width ≥ 8 mm = *T. latifolia*; gap present (>5 mm), leaf width < 8 mm = *T. angustifolia*; gap present (>5 mm), leaf width ≥ 8 mm = *T. x glauca*; unpublished data; Kuehn and White 1999; Kuehn et al. 1999; Bansal et al. 2019). We prioritized younger leaf tissue for collection (≥ 10 cm), placed it on ice, and then froze it once in the lab. For each of 39 populations, we collected 5 individuals that were at least 10–20 m apart (to minimize sampling clones). The number of individuals collected and the distance between them are well within the range of other molecular studies that have sampled between 3 and 9 cattail specimens that were 2–3 m apart (Kirk et al. 2011). For 4 out of the 39 populations, we only collected 1 plant specimen and for another population, we collected 4. Because our interest was in a broad geographic area of the Midwest, we traded-off number of individuals for a larger sampling area. We thus sampled 39 cattail populations in 7 states (Illinois, Indiana, Iowa, Michigan, Minnesota, South Dakota, Wisconsin) for a total of 178 individuals (Fig. 1, Table 1). Voucher specimens for 19 populations were collected and are archived at the Chicago Botanic Garden herbarium (CHIC Accession numbers 19,662 through 19,670 and 19,814 through 19,823).

In the lab, frozen plant tissue was ground with either dry ice or liquid nitrogen using a mortar and pestle. DNA extraction was done using Qiagen DNEasy Plant kits. To confirm successful DNA extraction, we ran genomic DNA samples in 0.5% agarose gel electrophoresis.

We carefully screened a total of 35 nuclear microsatellite markers that had been previously used in cattail species to determine which ones were able to discriminate between parental species and their hybrids in the geographic region we sampled. We initially tested 11 microsatellite markers derived from *T. angustifolia* (Tsyusko-Omeltchenko et al. 2003) because previous studies had found several to be species-specific for *T. latifolia* and *T. angustifolia* in North America (Snow et al. 2010; Travis et al. 2010; Kirk et al. 2011). However, out of the 6–7 markers that these studies found species-specific

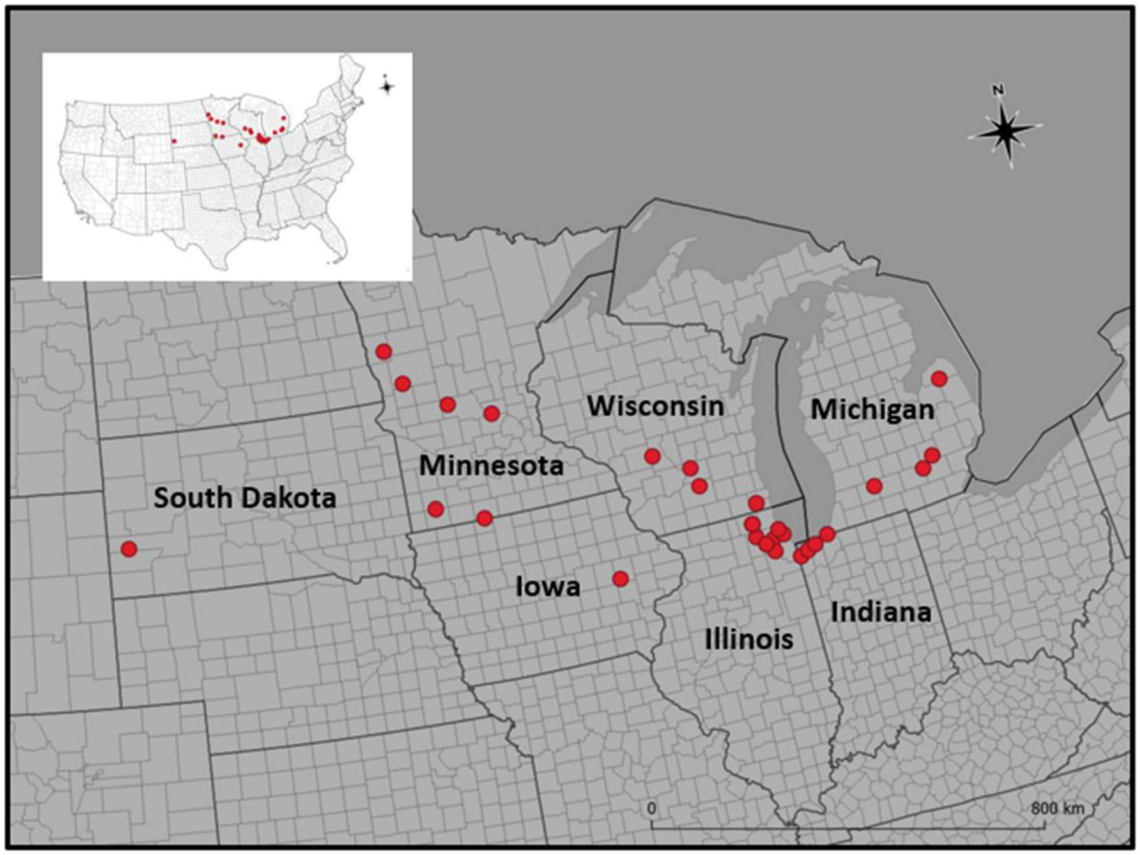

Fig. 1 Distribution of sampled populations across 7 states in the Midwest (QGIS Development Team 2020). Note: Some sampling locations have the same GPS coordinates and are thus not able to be distinguished (see Table 1)

(TA 3, TA 5, TA 7, TA 8, TA 16, TA 20, TA 21), we found only 4 to be species-specific in our samples (TA 3, TA 5, TA 8 and TA 16). We tested an additional 7 microsatellite markers from *T. latifolia* (Ciotir et al. 2013a), but, like the remaining TA primers from Tsyusko-Omeltchenko et al. (2003), none of them were useful in our study because they either failed to amplify or they overlapped in their size, preventing clear differentiation of parental species and making species diagnoses and hybridization characterization impossible.

Lastly, we screened an additional 17 microsatellite markers from *T. minima*, a congener native to Europe and Asia that does not occur in the Midwest (Csencsics et al. 2010), and we found 2 (TM 4 and TM 11) to be species-specific for *T. latifolia* and *T. angustifolia*. Each of these two TM primers gave two consistent, non-overlapping marker size patterns (one size from each parental species) and a signal for the hybrid exhibiting the two loci for each parental species. To determine which locus size corresponded to which parental species, we correlated the samples provisionally identified as *T. latifolia* morphologically with the two locus sizes for each TM primer. We found that 90% of the samples provisionally identified as *T. latifolia* correlated with loci sizes 193–198 bp for TM 4 and 305–307 bp for TM 11 (Table 2). Additionally, we found high degree of agreement between molecular identification using these two TM primers and the four TA primers previously deemed species-specific for the parental species (Snow et al. 2010; Travis et al. 2010; Kirk et al. 2011). Out of 51 samples that had provisionally been identified morphologically as *T. latifolia*, 42 samples (82%) showed perfect agreement between molecular identification as *T. latifolia* using the 2 TM primers and the 4 TA primers. For the 9 other samples, either one or two TA primers disagreed in the identification among the 6. Overall, all six markers used in this study (TA 3, TA 5, TA 8, TA 16, TM 4, and TM 11) consistently produced unique microsatellite lengths that were non-overlapping among species (Table 2) and were therefore deemed species-specific and used in all samples with the exception of TA 16, which did not amplify for 2 samples, and TM 11, which did not amplify for 4 samples.

Microsatellite primers were amplified using 2-step PCR (Schuelke 2000). The first PCR step allowed for the microsatellite primers (containing an M-13 tail of 18 nucleotides: TGTAAAACGACGGCCAGT) to anneal to cattail DNA whereas the second PCR step allowed attachment of a fluorescent dye (with the M-13 tail) to the amplified microsatellite regions for microsatellite sizing (Schuelke 2000). Step 1 PCR cycles were set to 94 °C for 3 mins, followed by 13 cycles of

Table 1 Sampling locations and year of collection for 39 cattail populations with corresponding GPS coordinates. Numbers in parentheses correspond to number of populations that have identical GPS coordinates because distance between them is below GPS minimum detection level

Location	Latitude (degrees)	Longitude (degrees)	Sampling year
Wood Dale, IL (2)	41.942545	−87.977977	2012
Darien, IL	41.728568	−88.009649	2012
Elgin, IL (2)	42.058209	−88.361985	2012
West Chicago 1, IL	41.89008	−88.162163	2012
West Chicago 2, IL	41.888897	−88.16283	2012
Chicago, IL	41.984109	−87.725174	2012
Glenview, IL (2)	42.088472	−87.814888	2012
Woodstock 1, IL (3)	42.291014	−88.365001	2012
Woodstock 2, IL	42.290988	−88.359694	2012
Woodstock 3, IL	42.288195	−88.35633	2012
Woodstock 4, IL	42.296384	−88.355349	2012
Freeborn, MN	43.717763	−83.088432	2012
Iona, MN	43.847711	−95.781922	2012
Hawley, MN	46.812224	−96.324463	2012
Ceylon, MN	43.513674	−94.630449	2012
Getty Township, MN	45.649502	−94.954827	2012
Fergus Falls, MN	46.192688	−95.995439	2012
Monticello, MN (2)	45.326159	−93.908019	2012
Keystone, SD	43.904178	−103.533943	2012
Cedar Rapids, IA	41.942766	−91.711308	2012
Portage, WI	43.540717	−89.431033	2012
Kansasville, WI	42.633554	−88.129828	2012
Windsor, WI	43.194864	−89.343922	2012
Camp Douglas, WI (2)	43.921	−90.261	2012
New Buffalo, MI	41.7646	−86.742	2013
Galesburg, MI	42.3108	−85.359833	2013
Brighton, MI	42.5052	−83.844183	2013
Waterloo Township, MI	42.34665	−84.1308	2013
Schererville 1, IN	41.520533	−87.456183	2013
Schererville 2, IN	41.520967	−87.455817	2013
Gary, IN	41.593151	−87.264366	2013
Porter, IN	41.658406	−87.062357	2013

Table 2 Allele fragment size ranges (in bold) and mean/mode of diagnostic primers found in this study compared with Tsyusko-Omeltchenko et al. (2003), Snow et al. (2010), Kirk et al. (2011), and Csencsics et al. (2010). It is worth noting that our results show sizes that are shifted towards larger values compared to other studies, likely as a result of the addition of the M-13 tail used in our 2-step PCR process (Schuelke 2000) that adds 18 base pairs to the marker sequence. L = *Typha latifolia*; A = *Typha angustifolia*

Primer	Repeat motif	This study		Tsyusko-Omeltchenko et al. (2003)		Snow et al. (2010)		Kirk et al. (2011)		Csencsics et al. (2010)
		L	A	L	A	L	A	L	A	
TA 3 (n=178)	(AG)	**185–197,** 194/193	**227–234,** 229/228	**177–211**	**177–231**	**174–180**	**210–216**	**172–184**	**213–221**	–
TA 5 (n=178)	(AG)	**293–299,** 297/297	**304–311,** 307/305	**282–296**	**282–308**	**276–282**	**286–294**	**275–285**	**282–292**	–
TA 8 (n=178)	(AC)	**286–292,** 289/288	**306–309,** 307/306	**270–280**	**268–296**	**267–271**	**273–291**	**268–270**	**274–289**	–
TA 16 (*n* =176)	(CT)	**195–198,** 195/195	**207–212,** 209/209	**183–201**	**171–229**	**167–179**	**191–195**	**178–192**	**190–194**	–
TM 4 (n=178)	(ACT)	**193–198,** 195/195	**188–190,** 189/189	–	–	–	–			**198–246**
TM 11 (*n* =174)	(ATA)	**305–307,** 306/306	**302–304,** 303/303	–	–	–	–			**296–302**

94 °C for 30 s, 57 °C for 1 min, 72 °C for 1 min and ended with 72 °C for 10 mins and a 10 °C continuous hold. Step 2 PCR cycles were set to 94 °C for 3 mins followed by 27 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min and ended with 72 °C for 10 mins and 10 °C continuous hold. After the 2-step PCR, we ran a 1.4% agarose gel electrophoresis to confirm successful microsatellite amplification.

Microsatellite analysis was performed on a Beckman Coulter gene sequencer and the sizing (400 bp ladder), scoring, and microsatellite interpretation were done using Beckman Coulter software. "Pure" parental species were inferred if all 6 diagnostic microsatellite markers (TA 3, TA 5, TA 8, TA 16, TM 4, TM 11) were in agreement in the molecular identification (for example, all microsatellites markers indicated a sample was a *T. latifolia* individual). F1 hybrids, *T. x glauca*, were identified if each marker showed one allele from each parental species. If the 6 markers revealed a mixture of hybrid and one parental species locus, then we identified those samples as backcrosses to that parent. Lastly, if the 6 markers revealed a mixture of hybrid and/or both parental loci, we classified these samples as advanced-generation hybrids (AGH) (Snow et al. 2010; Travis et al. 2010, 2011).

Results

Using the 6 diagnostic markers (4 from *T. angustifolia* that had been used previously and 2 novel ones from *T. minima*), we found that across all samples ($n = 178$), 25% were molecularly identified as *T. latifolia*, 6% as *T. angustifolia*, and 19% as *T.* x *glauca* (Fig. 2a). In addition, half of all samples (50%) were classified as advanced-generation hybrids (AGH) (Fig. 2a). Out of the AGH samples, more than half (58%) were backcrosses to *T. latifolia*, whereas 31% were backcrosses to *T. angustifolia* (Fig. 2b). For 11% of the samples, there was no clear direction towards either parent, as markers identified samples as either all three (*T. latifolia*, *T. angustifolia*, and *T. x glauca*, 1 sample) or as *T. latifolia* and *T. angustifolia* (9 samples) (Fig. 2b Unknown AGH).

The "pure" parental species were detected in only 49% of the sampled populations, while advanced-generation hybrids were found in 66% of the sampled populations. Moreover, there were only 7 out of the 39 sampled populations where all individuals tested in a population were identified molecularly as *T. latifolia*, whereas we found no population where all individuals tested were identified molecularly as *T. angustifolia*.

Agreement Between Morphological and Molecular Identification

For *T. latifolia*, 75% of samples exhibited agreement between morphological and molecular identification, while only 11% of *T. angustifolia* and 22% of *T. x glauca* samples showed

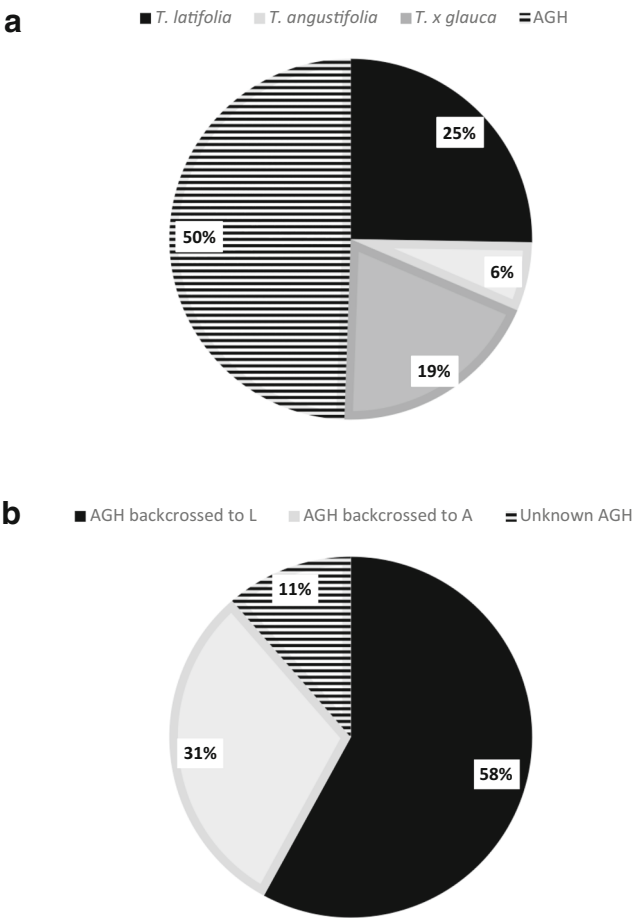

Fig. 2 **a** Molecular identity of cattail samples from 39 populations ($n = 178$). **b** Molecular identity of advanced-generation hybrids identified in (**a**). AGH = advanced-generation hybrids; L = *Typha latifolia*, A = *Typha angustifolia*

agreement between the two (Table 3). Out of 51 specimens morphologically identified as *T. latifolia*, 9 specimens were AGH (18%) and 4 specimens were *T. x glauca* (8%) when analyzed molecularly. There were no specimens that were morphologically identified as *T. latifolia* that were identified as *T. angustifolia* when analyzed molecularly (Table 3). Out of 95 specimens morphologically identified as *T. angustifolia*, 57 specimens were AGH (60%), 5 specimens were *T. latifolia*

Table 3 Agreement between morphological (first column) and molecular identification based on numbers of samples (percentages are in parentheses). Numbers in bold indicate the extent of agreement between morphological and molecular identification

	Molecular ID			
Morphological ID	*T. latifolia*	*T. angustifolia*	*T. x glauca*	AGH
T. latifolia	**38 (75%)**	0	4	9
T. angustifolia	5	**10 (11%)**	23	57
T. x glauca	2	1	**7 (22%)**	22

(5%) and 23 specimens were *T.* x *glauca* (24%) when identified molecularly (Table 3). Out of 32 specimens morphologically identified as *T.* x *glauca*, 22 specimens were AGH (69%), while only 2 were identified as *T. latifolia* (6%) and only 1 as *T. angustifolia* (3%) (Table 3). Although 50% of all samples were advanced-generation hybrids, none of them were identified as such using morphology, but once analyzed molecularly, 18% of morphologically identified *T. latifolia*, 60% of morphologically identified *T. angustifolia*, and 69% of morphologically identified *T. x glauca* samples were advanced-generation hybrids instead.

Discussion

Our molecular analyses revealed that six microsatellite markers allowed us to consistently discriminate between cattail parental species (*T. latifolia* and *T. angustifolia*), their first-generation (F1) hybrid (*T. x glauca*), and introgression including backcrosses to the parental species as well as advanced-generation hybrids in areas of the Midwestern US. Four of these microsatellites had been deemed species-specific to *T. latifolia* and *T. angustifolia* in southern Canada and in the US upper Midwest and northeast (Snow et al. 2010; Travis et al. 2010; Kirk et al. 2011). Our study, however, adds two more microsatellite markers from *T. minima* that are also species-specific for *T. latifolia* and *T. angustifolia*. To our knowledge, this is the first instance in which *T. minima* microsatellite markers have been found to be species-specific for *T. angustifolia* and *T. latifolia*.

Surprisingly, we found that the relative frequencies of parental species (*T. latifolia* and *T. angustifolia*) were relatively low whereas about half of the samples were advanced-generation hybrids. Although a larger sample size than in this study ($n = 178$) would allow for much more robust conclusions, our findings imply introgression is widespread in the area we sampled and much higher than had originally been reported for other areas of North America (Snow et al. 2010; Travis et al. 2010; Kirk et al. 2011; Marburger and Travis 2013). In addition, the relative abundance of the native parent (*T. latifolia*) was almost 4 times as high as the abundance of the exotic parent (*T. angustifolia*). In terms of relative frequencies, our values align well with those of Snow et al. 2010 for *T. latifolia* (25% this study; 27% their study) and *T. x glauca* (19% this study; 21% their study). However, our study revealed the relative frequencies of *T. angustifolia* were substantially lower (6%) than in their study (32%; Snow et al. (2010)). With respect to advanced-generation hybrids, our study revealed the highest relative frequency of AGHs compared to 4 other studies (Snow et al. (2010); Travis et al. (2010); Kirk et al. (2011); Freeland, Ciotir, and Kirk (2013; 2 different data sets)). Our study also showed the highest frequency of backcrosses to *T. angustifolia* (15.5%) and to *T. latifolia* (29%) compared to the above-mentioned studies (2–14% and 0–4%, respectively). Given the small number of primers used, we believe this is a conservative estimate of the frequency of AGH and should be viewed cautiously. Some AGHs may have been missed making the actual number possibly higher than reported.

We can think of two possible reasons for our higher frequencies of advanced-generation hybrids relative to previous studies. First, our study relied on a subset of molecular markers that were used in all the other studies, but we also included two novel markers from *T. minima* that none of the other studies utilized (TM 4 and TM 11, Csencsics et al. (2010)). Secondly, our results may be reflective of further progression of hybridization in cattail species in the area we sampled. Our sampling area is closest to the areas sampled by Snow et al. (2010) and Travis et al. (2010) which were sampled between 2004 and 2006, about 6–9 years before our sample collections. Lastly, because our samples may have come from more highly disturbed areas, such as areas along interstate highways, relative to more secluded areas for the other studies (e.g., federal lands in National Parks in Travis et al. 2010), this exposure to dispersal in highly trafficked areas may have resulted in increased opportunities for hybridization. However, further scrutiny of this claim is warranted given that a recent study showed that hybridization was comparable between wetlands and disturbed sites (Pieper et al. 2020).

Contrary to the findings of Snow et al. (2010), Travis et al. (2010), Kirk et al. (2011), and Freeland et al. (2013) who found that backcrosses to the exotic parent *T. angustifolia* exceeded those to the native parent *T. latifolia,* we found backcrossings of F1 hybrids to the native parent to be approximately twice as frequent as to the exotic parent (*T. angustifolia*). We hypothesize reasons for the bias in the direction of observed backcrossings to *T. latifolia* could result from an initial lower abundance of *T. angustifolia* as either a cause or consequence of reduced backcrossings to that parental species. Additionally, previous authors reported that cattail hybridization was overwhelmingly asymmetric with *T. angustifolia* providing ovules (i.e., maternal parent) and *T. latifolia* providing pollen (i.e., paternal parent; Ball and Freeland 2013; Pieper et al. 2017). Although they did not find flowering time in the parental species to be a pre-zygotic reproductive barrier to prevent hybridization because they overlapped in flowering times, Ball and Freeland (2013) strongly suggest that some barriers may exist that prevent *T. latifolia* female flowers being pollinated by *T. angustifolia*.

Our study thus suggests that the unprecedented high frequency of backcrossing events may be causing extinction by hybridization via genetic swamping of native *T. latifolia*. Although Travis et al. (2010) and Marburger and Travis (2013) discussed this possibility, their data showed much lower levels of introgression to *T. latifolia* than we found in this study (only 3% in Travis et al. (2010) vs. 29% in this study; no

data provided in Marburger and Travis (2013)). In genetic swamping, parental alleles may be conserved in the advanced-generation hybrids, but the pure parental species genotypes disappear (Levin et al. 1996; Rhymer and Simberloff 1996; Largiadèr 2007; Todesco et al. 2016). In their model, Huxel (1999) found that native taxa could be displaced by non-native ones relatively rapidly, and that due to high rates of globalization and the presence of cryptic species (sensu Morais and Reichard 2018), a characteristic that has been attributed to *Typha* species (e.g., Marburger and Travis 2013), extinction rates of native species could accelerate if hybridization is common. Additionally, McKenzie-Gopsill et al. (2012) and Pieper et al. (2018) found no niche segregation along a water-depth gradient for the two parental cattail species and their hybrid, suggesting all three compete for similar habitat (but see Grace and Wetzel (1981) and Travis et al. (2010)). Thus, loss of biodiversity at the expense of reduction in abundance of parental species is expected (Wolf et al. 2001). Because hybrid vigor has been documented for the F1 hybrid cattail (e.g., Travis et al. 2010; Bunbury-Blanchette et al. 2015; Zapfe and Freeland 2015), this reduction of the parental species is even more likely to occur.

Pieper et al. (2017) determined that due to asymmetric mating with lower introgression into the native parent as well as reduced hybrid fertility, *T. latifolia* should be favored in its maintenance over *T. angustifolia*. Yet our results point to opposite findings with higher introgression towards the native parent (*T. latifolia*) as opposed to the exotic parent (*T. angustifolia*). However, population dynamics of native *T. latifolia* should be studied further because differential seed set may mediate the likelihood for its extinction by hybridization. For example, when *T. x glauca* pollinates *T. latifolia*, seed set is almost zero, whereas when *T. latifolia* pollinates *T. x glauca*, seed set is relatively high (~1800 seeds/g; Pieper et al. 2017). In contrast, when *T. latifolia* pollinates *T. latifolia*, seed set is highest at ~2800 seeds/g (Pieper et al. 2017), allowing potential compensation for the almost zero seed set or lower seed set when introgression to *T. latifolia* occurs. These results and the context-dependency of which species provides pollen or ovules warrant further and urgent examination of the potential future of native *T. latifolia* and its vulnerability to extinction by hybridization, given that such an outcome has already been documented in a very similar system with *Spartina* species (Ayres et al. 2004). Because hybridization seems to be happening quite rapidly, managers could resort to pre-emptive seed banking for *T. latifolia* to at least try to preserve the native species and, more widely, cattail genetic diversity.

Performing molecular analyses on morphologically identified specimens allowed us to conclude that using leaf width and gap between inflorescences, traits widely used in the field, is not a robust technique to reliably identify the parental cattail species or their hybrids (both F1 and AGH). In addition, we found that the level of agreement between molecular and morphological identification was species-dependent. For example, the native cattail (*T. latifolia*) exhibited the highest level of agreement between molecular and morphological identification using those two morphological traits at 75% of samples, meaning that we were wrong in our morphological ID in 25% of samples. In contrast, *T. angustifolia* exhibited the lowest degree of agreement at only 11%, whereas the F1 hybrid, *T. x glauca*, showed intermediate levels of agreement (22%) between molecular and morphological identification. Kuehn and White (1999) showed high correlation (~90%) between morphological and genetic identification of cattails, with highest correlations found for *T. latifolia* (95%), followed by *T. angustifolia* (93%), and *T. x glauca* (83%), but the genetic markers used (RAPDs) have presented long-standing concerns regarding the need for many loci to identify backcrosses and advanced-generation hybrids and their repeatability from lab to lab (Waldron et al. 2002; Selkoe and Toonen 2006; Novello et al. 2010), potentially undermining these results. Using microsatellite markers, Kirk et al. (2011) also found good correspondence between molecular ID and morphological identification based on leaf width, but, contrary to this study, the occurrence of advanced-generation hybrids in their study was lower than that of F1 hybrids. Our study revealed that half the samples we collected were AGH, highlighting how misleading morphological identification alone can be, agreeing with previous claims (Travis et al. 2010; Marburger and Travis 2013). We thus urge caution when using these two morphological traits alone to identify cattail species and advocate for a combination of morphological as well as molecular approaches when feasible.

Cryptic invasions, extremely common in hybridizing species like *Typha* (Marburger and Travis 2013), make managing and eradicating them problematic because identification of parental species and hybrids is difficult and usually requires molecular tools. Furthermore, hybrids tend to possess traits that make their management even more challenging (e.g., fast growth rates, clonal expansion, asexual and sexual reproduction, high seed production, easy and widespread seed dispersal (e.g., by wind), highly adaptable, etc. (Morais and Reichard 2018; Bansal et al. 2019)). Working on rapid detection of hybridization and fast responses to mitigate such invasion events are an ideal proposition, yet the vigilance required to do so is logistically and economically impractical for many managers, who tend to be short-staffed and depend on low and fluctuating sources of funding. Although we advocate rapid detection and mitigation of invaders, including exotic and hybrid cattail, we know this may not always be realistic. Since extinction by hybridization is generally predicted to become an even more severe problem in a warmer, more globalized world where species boundaries shift and overlap, future research should strongly prioritize how this threat will affect *T. latifolia* and, more generally, native species that are predisposed to hybridization.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability (Software Application or Custom Code) Not applicable.

Declarations

Ethics Approval Not applicable.

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